GLYCAN ANALYSIS OF PROTEINS AND CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/679,202, filed Jun. 1, 2018, the contents of which are incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. R21CA225474 provided by the National Cancer Institute. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Changes in the N-linked glycosylation of cells surface and secreted proteins are known to occur in many cancers. Indeed, these glycans often mediate the interactions between the cancer cell and its environment. Almost every currently used biomarker for cancer is either a glycoprotein, such as carcinoembryonic antigen (CEA) or a glycan itself, such as CA-19-9. However, due to the inherent difficulty in glycoproteomics, where the identity of a protein and the glycans on that protein are deduced, most glycoprotein biomarker assays target either just the protein itself, such as is the case with CEA, or just the glycan itself, which is the case with CA-19-9. Recent work has shown that specific glycans on specific proteins can act as biomarkers of cancer and often they are better markers than the protein alone. However, obtaining this glycan information is laborious and difficult.

[0004] Currently, glycans are examined on either individual proteins or in large protein pools, such as serum or urine, where glycan information is obtained but protein information is lost. Thus, the tradeoff is that glycan information can be obtained for a few proteins analyzed one by one with site of glycan attachment, or data can be obtained for groups of proteins or glycans (but not both together). One approach that attempts to address this issue is the use of antibody lectin arrays. In this case, antibodies to specific proteins are spotted onto glass slides and the glycans on the captured glycoproteins are interrogated with sugar binding proteins (lectins). While this data does provide evidence for specific structural motifs, it offers no true insight into the glycan diversity on a protein nor does it offer true structural information.

[0005] There remains a need for a method to deliver structural glycan information for specific glycoproteins in complex solutions. The present invention satisfies this need.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides a method for glycan analysis of at least one sample, the method comprising the steps of: providing a substrate having a surface spotted with a plurality of antibodies; incubating the substrate in a blocking solution; incubating the substrate in at least one sample; spraying the substrate with an enzymatic releasing solution; and scanning the substrate by mass spectrometry to detect and identify the presence of glycans.

[0007] In one embodiment, the at least one sample comprises at least one protein solution. In one embodiment, the at least one sample comprises at least one population of cells. In one embodiment, the at least one population of cells is incubated in a fixing and rinsing agent prior to the step of spraying the substrate with an enzymatic releasing solution. In one embodiment, the fixing and rinsing agent is selected from the group consisting of: formalin, Carnoy's solution, paraformaldehyde, an ethanol-based fixative, and a polyethylene glycol-based fixative.

[0008] In one embodiment, the substrate is a glass or plastic microscope slide or multiwell plate. In one embodiment, the blocking solution is a serum. In one embodiment, the serum is 1% BSA in PBS and detergent. In one embodiment, the blocking solution is removed with a wash step comprising 3×PBS baths and 1× water bath. In one embodiment, the at least one sample is incubated in a humidity chamber at room temperature for two hours. In one embodiment, the enzymatic releasing solution comprises PNGase F.

[0009] In one embodiment, the mass spectrometry is selected from the group consisting of: matrix-assisted laser desorption/ionization imaging Fourier transform ion cyclotron resonance (MALDI-FTICR) mass spectrometry, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry, scanning microprobe MALDI (SMALDI) mass spectrometry, infrared matrix assisted laser desorption electrospray ionization (MALD-ESI) mass spectrometry, surface-assisted laser desorption/ionization (SALDI) mass spectrometry, desorption electrospray ionization (DESI) mass spectrometry, secondary ion mass spectrometry (SIMS) mass spectrometry, and easy ambient sonic spray ionization (EASI) mass spectrometry. In one embodiment, the scanning step is preceded by a step of spraying the substrate with a MALDI matrix material. In one embodiment, the MALDI matrix solution is selected from the group consisting of: 2,5-dihydroxybenzoic acid, α-cyano-4-hydroxycinnamic acid, sinapinic acid, 1,5-diaminonaphthalene, and 9-aminoacridine.

[0010] In one embodiment, the plurality of antibodies specifically bind to a protein selected from the group consisting of: A1AT, fetuin-A, hemopexin, Apo-J, LMW Kininogen, HMW Kininogen, apo-H, transferrin, IgG, IgM, IgA, fibronectin, laminin, ceruloplasmin, fibulin, angiotensinogen, Fibrillin-1, TIMP1, thrombospondin 1, galectin-3 binding protein, complement C1 R, clusterin, galectin 1, alpha-2-macroglobulin, Vitamin D binding protein, histidine rich glycoprotein, histidine rich glycoprotein, CD109, CEA, Cathepsin, AFP, GP731, and combinations thereof. In one embodiment, the antibodies are useful in detecting the presence of hepatocellular carcinoma.

[0011] In another aspect, the present invention provides a method for glycan analysis of at least one population of cells, the method comprising the steps of: adhering at least one population of cells to a surface of a substrate; fixing and rinsing the at least one population of cells; spraying the substrate with an enzymatic releasing solution; and scanning the substrate by mass spectrometry to detect and identify the presence of glycans.

[0012] In one embodiment, the at least one population of cells is adhered by culturing, deposition, swabbing, smearing, or centrifugation. In one embodiment, the fixing and rinsing agent is selected from the group consisting of: formalin, Carnoy's solution, paraformaldehyde, an ethanol-based fixative, and a polyethylene glycol-based fixative.